FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 4, ISSUE 3 ■ MAY-JUNE 1996

GENETICS RESEARCH ON STORED TISSUE: TWO PERSPECTIVES

Rapid advances in molecular genetics have raised new questions about the use of human cells and tissue in research—questions that have prompted several groups of leading scientists, clinicians, and bioethicists to take a second look at the issues surrounding the research use of stored tissue samples.

Some of the answers that those groups have come up with, most notably in the form of a model "Genetic Privacy Act" and a set of recommendations published in the *Journal of the American Medical Association (JAMA),* have sparked heated debate in the biomedical research community, and NIH is no exception. To shed light on this complex topic, *The NIH Catalyst* asked Leslie Biesecker, a medical geneticist at NCHGR, and Mark Sobel, a molecular biologist at NCI, to share their perspectives.

Before jumping into the fray, consider this background. A project funded by the Human Genome Project's Ethical, Legal and Social Implications (ELSI) branch kindled the controversy last year with the release of a report that included a proposal for a far-reaching piece of federal legislation called the Genetic Privacy Act. Another group, consisting of participants in a workshop convened by NCHGR and the Centers for Disease Control and Prevention (CDC), sharpened the focus of the debate with its consensus statement, "Informed Consent for Genetic Research on Stored Tissue Samples," in the Dec. 13, 1995, issue of *JAMA.*

Both supporters and critics of those reports agree that genetic tests can furnish valuable information that can improve the health of an individual. However, casting a pall across these positive aspects are some negative factors. Release of genetic test information may adversely affect patients and their

continued on page 14.

VETERINARY RESOURCES PROGRAM: STEERING NIH'S ARK INTO NEW WATERS

by Rebecca Kolberg

There's no room for more animals. It costs too much. All it does is provide housing. Those are just a few of intramural scientists' perceptions—and misperceptions—about NIH's Veterinary Resources Program that the center is working hard to change.

C. Max Lang, who took the helm of the Veterinary Resources Program (VRP) last October, doesn't fault researchers for expecting VRP to do a better job than it has in the past. "Our primary goal should be to provide the most humane animal care and the highest quality service at the lowest possible price," he says.

Bill Branson

Currently, VRP cares for about 75% of NIH's research animals—about 25,000 rodents, 500 rabbits, 400 pigs and ungulates, and 1,500 nonhuman primates—at 19 buildings in Bethesda and nearby Poolesville, Md. The remainder of the animals are cared for at institute, center, and division facilities or at contractors' facilities.

Upon arriving at NIH from Pennsylvania State University's Animal Resource Facility in Hershey, Lang found that his calculations supported intramural scientists' claims that VRP costs were steeper than at comparable biomedical research institutions. He also discovered that VRP had space-allocation policies that created a shortage of space for some species of animals, such as nonhuman primates, while they created an excess of space for others, such as rabbits. As part of an attempt to address both of those problems, Lang has redeployed staff and also changed VRP's practice of dedicating each building to a single species.

"We have room now. We want to get the word out to scientists that they can come back," says Lang, noting that many intramural researchers had turned to off-campus contractors for animal care when they ran into space problems at VRP.

continued on page 12.

CONTENTS

THE COMMUNITY CONNECTION: WHERE WE'VE BEEN AND WHERE WE'RE GOING

Brooks-Glogua

Janyce Hedetniemi

A scant 18 months ago, the NIH Office of Community Liaison was born amid frequent—and often angry—headlines in local papers that chronicled NIH's inadequacies as a neighbor and steward of the environment. The office has quickly come of age, aided by the intramural scientists who played an important role in NIH's first wave of response to community criticism.

Our whirlwind accomplishments include conducting soil tests on the campus, forming an ad hoc group of scientists and technicians that found that NIH's incineration of medical and pathological waste has not had a negative impact on the environment and neighborhood, revising the NIH Bethesda Campus Master Plan, and establishing a structure for information exchange with our neighbors.

The impetus for these changes stems from a May 1994 meeting at which NIH Director Harold Varmus and community leaders negotiated several agreements. At the time, the most pressing community concern was NIH's incineration of medical pathological waste on campus—a practice that was permanently halted shortly after the meeting. That action left NIH with a pressing need to find alternative ways to handle such waste and to reduce its volume.

The Environmental Concerns Working Group was formed to address that and other problems. NHLBI's Hank Fales chaired a subgroup on alternative waste strategies that through research, field trips, and interviews completed a report on requirements for an acceptable on-campus technology for waste disposal that will be indispensible as we plan for the future. A recycling subgroup, headed by NCI's Kira Lueders, developed a blueprint for a campus-wide recycling program and implemented voluntary and interim programs that include recycling of pipet-tip racks, white paper, and aluminum cans in some buildings. NIDDK's Jane Sayer chaired a subgroup that took on the formidable task of developing procedures to replace paper catalogs with electronic ordering systems, tighten procedures for ensuring correct mail delivery, and reduce paper use in laboratories and offices.

Hand in glove with concerns about incineration was the community's fear that NIH's past incineration had harmed the environment and neighborhood. To address those worries, NIH worked with the community to test 45 sites on campus in accordance with the Environmental Protection Agency's standard protocols. A panel of 12 nationally renowned scientists and technicians was convened to evaluate the soil-testing results and assess the impact of those results on the campus and neighborhood. The experts' findings were expected to be available by the end of April.

Perhaps the most rewarding activities have resulted from NIH's commitment to listen more closely to the community, to revise the content of the NIH Master Plan, and to be more sensitive to the impact of traffic, construction, noise, and pollution.

Since the 1994 accord, better and more explanatory signs about construction projects have become standard across campus. Community briefings were held on sensitive subjects such as plans to reduce emissions from the boilers and reduce noise from the chillers in the Power Plant in Building 11. Neighbors also worked with NIH to mitigate noise and light from Multi-Level Parking Lot-8 by installing louvers and landscaping.

The revision of the draft NIH Master Plan resulted in a remarkable partnership. For more than a year, my office and other NIH staff and consultants worked closely and regularly with a Master Plan Community Group composed of representatives from 30 neighborhoods, the National Capital Planning Commission, the Maryland National Capital Parks and Planning Commission, the Bethesda Chamber of Commerce, and the Montgomery County Council and government offices. After extensive public scrutiny and review, the National Capital Planning Commission approved the NIH Master Plan on Feb. 1, citing the plan and our outreach to the community as a model for other federal agencies to follow.

Despite these impressive strides, much remains to be done. Acting on behalf of the Office of the Director and the entire NIH community, the Office of Community Liaison will continue to promote the policy of openness and collaboration as the new Master Plan is implemented. It is also my hope that we can extend many of our resources to our neighbors, such as improving the ways we share information on health promotion, disease prevention, and science education. Today, NIH is an unequaled *national* resource. In the future, I hope it will also be renowned as a unique and positive *community* resource.

To help achieve this goal, intramural researchers can send their comments and suggestions to me at the Office of Community Liaison (phone: 496-3931; fax: 594-2592; e-mail: hedetnjn@od1tm1.od.nih.gov). ■

Janyce Hedetniemi
Director
Office of Community Liaison

CATALYTIC REACTIONS

Below are comments that we received for topics that were raised in the March-April issue.

On scientific job openings

The NIH tenure track system was revamped last year, and the new staff scientist track was created. Such positions were supposed to be open-application to remove the old-boy network that used to exist at NIH. But as we all know, there are ways around every system. How many of these advertised positions are "real," and how many are carefully tailored for a particular candidate from the lab itself? Some of the job ads seem to be ridiculously specific—just pick up some of the weekly job announcements for scientists at NIH and it's obvious some are designed to exclude all but a particular person. If nothing's really changed, why pretend it has? It's still an old-boy network.

—*Anonymous*

When an institute decides it needs to find a person to fill a programmatic need in a tenure-track slot, there are often candidates within the lab who apply for the job. The heterogeneous composition of the search committee guarantees that neither the lab or branch chief nor the scientific director will guide the search, and in all cases, multiple candidates for the position are reviewed and interviewed. Sometimes the "inside" candidate, if there is one, is best qualified, sometimes not. With respect to this being an "old-boy network," fully 27% of the candidates found by searches for NIH tenure-track positions have been women. The best evidence that the program is more open is that since the search process was mandated, nearly half of recruits to tenure-track positions at NIH have come from outside institutions and another 18% trained at a different NIH institute than the one offering the job. ■

—Michael Gottesman, DDIR

Glycoday, Act II

The second annual Glycoday celebration, hosted by NIH's Glycobiology Interest Group, will be held May 28 at the Holiday Inn in Annapolis, Md. There is no registration fee, but interested parties must preregister by May 21. For more information, contact Diana Blithe (phone: 496-6437; e-mail: blithed@cc1.nichd.nih.gov).

Just Ask!

Dear Just Ask:
Consider the following incident. It is 10:30 a.m. on Saturday, and I am in my lab working. A man wearing an NIH I.D. badge displayed prominently from a necklace passes me in the hall. I nod to him, and return to my work. Five minutes later, the man—who turns out to be an NIH health physicist—approaches me and admonishes me for "not challenging him."

What defines an appropriate "challenge?" If I did not adequately "challenge," my reasons for not doing so were that 1) he had an NIH I.D. badge prominently displayed, 2) I judged him, by his appearance, to be a person who had some business being in the lab, 3) my natural instinct is to be courteous, not confrontational, and 4) he was a big guy—maybe a foot taller and at least 50 pounds heavier than me. I am 5 foot 2 inches tall.

Practically speaking, I am not sure how to react to any situation—especially an adverse one—that a stranger might present. I recognize that we all must contribute to NIH security. However, might trying to force NIH staff to provocatively encounter strangers end up endangering the staff themselves—most of whom are untrained in policing procedures or even self-defense?

—*Kuan-Teh Jeang, NIAID*

Dear K.T.:
Everyone around here—especially those of us who are on the short side—agrees that you have raised a very important question: what constitutes an appropriate "challenge" of interlopers?


Lorna Heartley

Celia Hooper

Debbie Thomson, acting chief of the Crime Prevention Branch of NIH's Division of Public Safety, suggests approaching strangers and asking them, "May I help you?" She says that's usually enough to constitute a reasonable challenge without making either the challenger or the person being challenged feel uncomfortable. If you spot someone who is behaving suspiciously or who intimidates you in any way, call security—115—immediately and leave the challenging to NIH police. When in doubt, Thomson says it's always better to err on the side of caution and call the police.

NIH's Radiation Safety Officer, Bob Zoon, adds that scrutiny by the Nuclear Regulatory Commission (NRC) has been the driving force behind some changes in the very open atmosphere that once pervaded NIH labs. "There is such trust here that someone whom no one knows could walk all around a lab, possibly even grab stuff out of a fridge and disappear—and no one would say anything to them. That's what the NRC really has a problem with," says Zoon, acknowledging that the policy of asking NIH staff to challenge strangers treads a very fine line. His office wants to heighten the NIH staff's awareness of security but does not want or expect such challenges to be confrontational or rude, especially if they involve legitimate visitors, health physicists, or even NRC inspectors. Although it isn't reasonable to expect NIH staff members to challenge every stranger in a public corridor, particularly in the Clinical Center or in non-lab areas such as Building 1 or 31, Zoon says that every unescorted stranger in a lab—especially in labs with restricted access or where radionuclides are used—should be gently challenged, even if they have an NIH I.D. Zoon recommends challenging with one of these lines: "I'm sorry, but I don't recognize you. Can I help you?" or "Are you visiting someone in the lab?" If a stranger turns out to be a visitor who is truly lost, he or she should be escorted out of the restricted area and directed to the correct destination. To help researchers grow accustomed to the practice of challenging strangers, Zoon has asked health physicists to inspect labs outside their assigned areas. "The idea is to assure NRC that unauthorized people do not have access to radioactive materials," he says, "You cannot just ignore someone that you do not know who is wandering around where they shouldn't be." ■

—C.H.

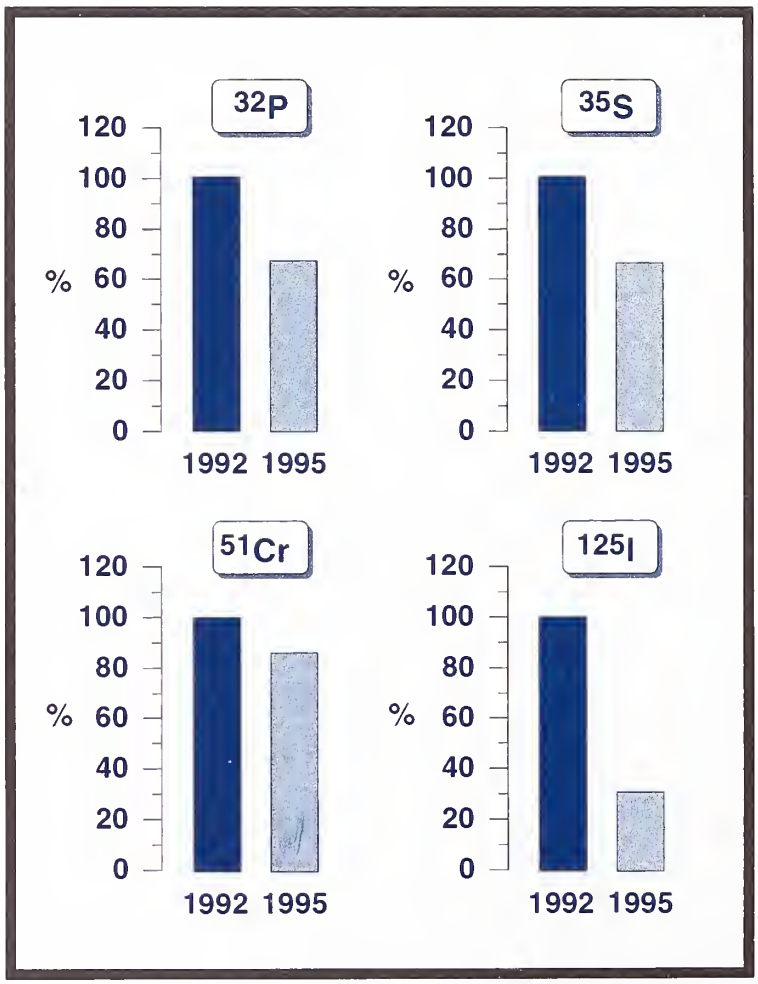
ALTERNATIVES TO RADIOACTIVITY IN BIOMEDICAL RESEARCH

by Juan S. Bonifacino, Ph.D., NICHD

The adoption of stricter laboratory security policies in response to heightened oversight by the Nuclear Regulatory Commission has changed the way research is done at NIH. Carrying our keys at all times has become a fact of life, and we have all had to adjust to the new reality of locked laboratories and freezers as well as to a higher level of scrutiny on the part of the NIH Radiation Safety Branch.

These requirements have also compounded the effort and costs associated with the use of radioisotopes in research. Most researchers may not be fully aware that in addition to the actual purchase price of radioactive materials paid by individual laboratories, there are many hidden costs paid for with NIH institutional funds. For instance, in 1995 alone, the cost of radioactive waste disposal at NIH was more than $4 million. When you figure in all the other costs in time and dollars—such as processing orders, keeping records, surveying for contamination, training and monitoring personnel—the total cost for the use of radioisotopes at NIH is staggering.

One of NIH's responses to the growing constraints on the use of radioactive materials has been to assemble a group of NIH scientists to identify, evaluate, and promote suitable nonradioactive research methods as alternatives to the use of radioactive materials. The mission of this Committee on Alternatives to Radioactivity will be greatly aided by two favorable trends. First, the use of many common radioisotopes at NIH has declined substantially in the past four years (see figure). Second, nonradioactive methods are becoming increasingly powerful, to the point that some have already gained widespread acceptance. The success of chemiluminescence-based detection systems for western blotting, for example, demonstrates clearly that when a nonradioactive alternative is superior to its radioactive counterpart, the technique is readily adopted. The committee hopes to accentuate these trends by improving access to information on novel nonradioactive alternatives and by providing incentives for using them.



Use of common radioisotopes at NIH.

The first activity organized by the committee will be a one-day workshop and technical exhibit on May 31. The workshop will be held at Masur Auditorium in Building 10 and will consist of scientific presentations on the principles and applications of nonradioactive alternatives to many experimental procedures used at NIH. The morning session will be devoted to nucleic acid labeling and detection techniques, including Southern and northern blotting, in situ hybridization, and SSCP analysis. The afternoon session will include talks about in vitro and in vivo phosphorylation techniques, cell-surface and total-protein labeling, in vitro translation, and emerging technologies. The speakers will provide a critical assessment of the value of the different methods in terms of sensitivity, accuracy, availability, ease of use, and cost. In conjunction with the scientific presentations, there will be a technical exhibit in Building 10's Visitor Center. Representatives from approximately 30 manufacturers of reagents and equipment will distribute literature and be available for consultation on the use of nonradioactive methods. The committee strongly encourages all NIH scientists who use radioactive materials to attend this meeting and to be ready to discuss their own successes and problems with using nonradioactive methods.

We are convinced that decreasing our reliance on radioactive methods—although it will take some time and effort—will lead to better working conditions at NIH. Remember, a laboratory with no radioactive materials does not have to be locked all the time! ■

LAND OF RISING SCIENCE: NEW FELLOWSHIPS PROMOTE JAPAN-U.S. INTERACTIONS

by Rebecca Kolberg

As part of a move to be an equal partner in the international biomedical research community, the Japanese government is launching an initiative to support Japanese fellows at NIH and to fund U.S. researchers' visits to Japan.

The Japan Society for the Promotion of Science (JSPS), as part of an arrangement developed with NIH's Fogarty International Center (FIC), announced plans on Feb. 9 to establish a program aimed at promoting Japanese-U.S. scientific exchange. The new program follows a pilot program, instituted last winter, that is currently supporting 30 Japanese fellows in their final year of training at NIH and 25 U.S. scientists, including eight NIH researchers, on short-term visits to Japanese labs. Under the new program, Japanese fellows at NIH will receive funding from JSPS for up to two years. JSPS, which is a branch of Japan's Ministry of Education, Science, Sports, and Culture, expects to award 30 of the competitive fellowships in 1996 and another 30 in 1997.

"The primary incentive to apply for this funding is that the institute, center, or division may not otherwise be able to accommodate such a postdoctoral experience. Plus, there's the prestige and the slightly higher stipend [than a regular visiting fellow]," says Associate Director for Intramural Affairs Philip Chen.

One of this year's recipients, Makoto Migita of NINDS, says he thinks the JSPS program is an excellent idea. "I was happy to get this funding so I could continue my research," says Migita, who has been at NIH about 2 1/2 years and will stay about seven months longer on JSPS funding. The M.D.-Ph.D., who is working on developing gene-therapy strategies for Gaucher's disease, says that when he returns to Japan he hopes to contribute to the development of gene-therapy research, an area in which he says his homeland currently lags behind Western nations.

> "I THINK THAT THE HIGHEST LEVELS OF THE JAPANESE GOVERNMENT HAVE DEVELOPED AN APPRECIATION FOR HAVING A WORLD-CLASS BASIC RESEARCH STRUCTURE..."
> —*Michael Snyder, FIC*

Michael Snyder, FIC's program officer for Japan and China, says, "I think that the highest levels of the Japanese government have developed an appreciation for having a world-class basic research structure, especially a life-sciences component ... and have realized that in order to do that, they need to be able to attract U.S. researchers to Japan and to support Japanese researchers around the world."

Lorna Heartley

Makoto Migita

In addition to the Japanese fellows program, JSPS will continue to fund a "limited number" of fellowships for U.S. researchers in the biomedical and behavioral sciences who want to pursue collaborative research at Japanese universities and scientific institutions. Migita is also enthusiastic about that part of the program, saying he hopes the exchange will broaden the scientific horizons of young Japanese researchers and make them more open to international collaborations.

Among the U.S. scientists going to Japan this year courtesy of JSPS is Susan Garges of NCI's Laboratory of Molecular Biology, who is spending a couple of weeks in the lab of a world-renowned expert on RNA polymerases, Akira Ishihama, at the National Institute of Genetics in Mishima. Garges wants to learn how to purify mutant RNA polymerases for her studies of transcription in *Escherichia coli*. "I had known that I'd probably have to go to Japan to do this work," she says, "When the JSPS announcement came along, it seemed like an ideal opportunity."

NIA's Edward Spangler also decided to get a taste of scientific life in Japan. He will work for four months with Hideki Kametani, a former colleague in NIA's Laboratory of Cellular and Molecular Biology, learning an innovative microdialysis procedure for the in vivo assessment of dopamine release in the brains of aging animals. Kametani's lab is at Fukuoka Prefectoral University on the southern Japanese island of Kyushu. Like Garges, Spangler doesn't know any Japanese, but in preparation for his trip, he picked up a few "survival words" from some Japanese postdocs at NIA.

Also heading off to the island of Kyushu is NCI's Angela Manns, whose epidemiological research centers on a retrovirus endemic to the Caribbean and Japan, human T cell lymphotropic virus-type 1 (HTLV-1). During her three-week stint with Shunro Sonoda, a Kagoshima University researcher with whom she has collaborated for several years, Manns hopes to gain some new insights into how immunogenetics influences the type of disease seen in HTLV-1 patients. She will also spend one week at the National Institute of Genetics' DNA research center.

The deadline for the next round of JSPS fellowships has not been set, but it is likely to be in late fall. For more information, contact FIC's Division of International Relations (phone: 496-4784, fax; 480-3414; e-mail: snyderm@nih.gov). ■

Catalyst Mailing List

To be added to or deleted from the *Catalyst* mailing list, or to change your mailing address, contact our editorial offices (phone: 402-1449; fax: 402-4303; e-mail: catalyst@od1em1.od.nih.gov).

Internet Grateful Med: Making a Good Thing Better

Access to online information is extraordinarily useful to biomedical researchers—and thanks to a new development, it's now even simpler to access one of the world's largest and most-used scientific databases, the National Library of Medicine's Medline.

Grateful Med, the software currently used by more than 90,000 subscribers to reach Medline and other National Library of Medicine (NLM) databases, is gaining a sidekick on the World Wide Web, appropriately called Internet Grateful Med. Set for production release in April, Internet Grateful Med is already netting positive reviews from NIH's WorldWideWeb Interest Group [see box below] and from intramural researchers who were given early access to the final testing, or "beta," version.

Original vs. Internet

Internet Grateful Med is both easier to use and more powerful than the original Grateful Med program. Unlike its forerunner, the initial version of Internet Grateful Med conducts searches only in Medline. However, NLM is already testing a follow-on version that searches several additional databases. Internet Grateful Med has better capabilities for picking and choosing records for output. You can print, save, or e-mail results using normal Web browser functions. Context-sensitive online help is available throughout the program. The original Grateful Med came in PC (DOS) and Macintosh versions. In contrast, Internet Grateful Med can be used from any computer with Internet access and a compatible Web browser, including Unix workstations. You can even access it using the Lynx character-mode browser from a dumb terminal!

In its current form, Internet Grateful Med helps the user create, submit, and refine a search in Medline. The user can search by subject, by text word in the title, or by author name. Searches can be limited by language, publication type, study group, age group, or range of years back to 1966. Internet Grateful Med offers direct links to the full text of Clinical Practice Guidelines supported by the Agency for Health Care Policy and Research and to nearly 60,000 online images from NLM's History of Medicine Division. Searching in additional databases will be available soon.

As does the original software, Internet Grateful Med includes the capability, called Loansome Doc, for requesting a hard copy of documents through the Interlibrary Loan process. However, unlike the original Grateful Med, it is not yet set up to download results in the tagged Medline format that some researchers use to import citations into bibliography programs such as Endnote or Reference Manager.

Another key difference is that the original Grateful Med allows the user

Getting Started

Ready and raring to go? Here's a checklist of what you need to operate Internet Grateful Med.

1. A Medical Literature Analysis and Retrieval System (MEDLARS) account from the National Library of Medicine (NLM). This account will give you a user identification and password for searching Medline and other NLM files. If you have an NIH Library Card or are qualified to get one, you are also qualified for a MEDLARS account. See the Main Desk at the NIH Library in Building 10 for an application form for the Library Card, or bring your Library Card to the desk.

2. A Web browser program. Of the graphical browsers suggested for use at NIH, the developers of Internet Grateful Med strongly recommend Netscape Navigator version 2.0 or higher for Macintosh computers or PCs running Microsoft Windows and NCSA X Mosaic version 2.6 or higher for UNIX workstations. One important note: the Windows and Macintosh versions of NCSA Mosaic do not work properly with Internet Grateful Med. If you have an account with NIH's Helix mainframe computer, you can use the Lynx command-line browser program by typing in "lynx" when you see the prompt "helix%".

3. An Internet connection. This can be either a direct connection via NIHnet or a remote connection via modem through a local Internet service provider (ISP) or through DCRT's Parachute system.

4. The correct Web address, or Uniform Resource Locator (URL), to access Internet Grateful Med: http://igm.nlm.nih.gov/ ■

WIGging Out

The WorldWideWeb Interest Group (WIG), just one of the dozens of interinstitute interest groups at NIH, is open to anyone interested in Internet issues. In addition to offering talks and discussions on topics of general interest, the group also features presentations on specific topics of interest to scientists, information providers, and technical users. Meetings are held on the second Tuesday of each month at 2:30 p.m. in Building 10, Lipsett Auditorium. For more information, see WIG's home page on the Web, located at http://mantis.dcrt.nih.gov/WIG/ ■

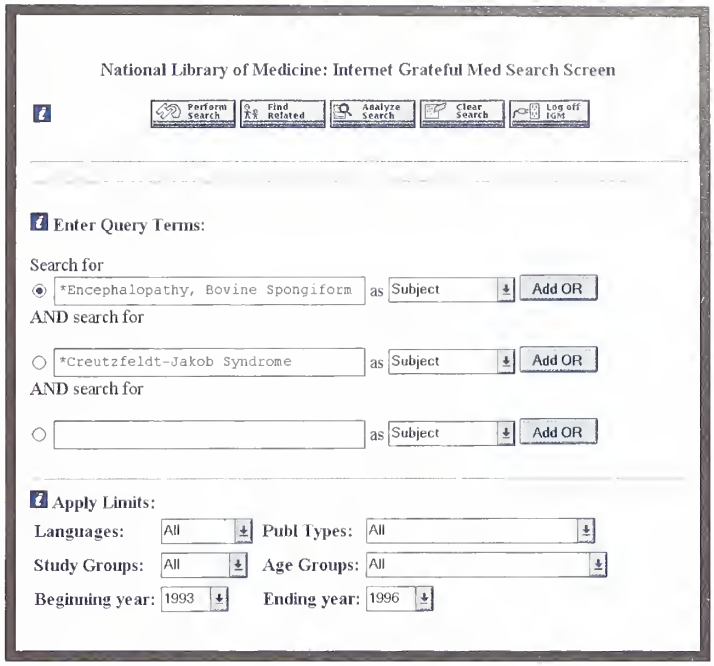
by Lawrence Kingsland, Ph.D., NLM,
and Dale Graham, Ph.D., DCRT

to save search strategies for later reuse, whereas Internet Grateful Med does not. As a Web-based application, Internet Grateful Med cannot read from the users' local computer disk. Small applications, or "applets," written in the Java programming language may provide a solution to this dilemma in the future.

How It Works

Internet Grateful Med has two major sets of assisted Medline search functions: "just do it" and "user invoked." "Just do it" functions are performed automatically in the background. "User invoked" functions involve situations in which the user is asked to clarify his or her search or to choose among suggested options. One of the "user invoked" functions offers the opportunity to restrict retrieval to articles in which a given term is a central concept of the article, and it offers guidance in adding subheading qualifiers to help focus a search.

Internet Grateful Med also provides a sophisticated "analyze search" function, which offers users the opportunity to substitute terms or add related terms to augment a search. This function also helps to clarify ambiguous terms such as "management," which to one user might mean "organization and administration" and to another, "therapy." Judicious use of the "analyze search" function can sometimes dramatically improve a disappointing initial search.



Although the graphical nature of most Web browsers makes Internet Grateful Med a snap for researchers to use [see "Getting Started," page 6], another feature of the World Wide Web posed major headaches for Internet Grateful Med's developers at NLM. The standard Web interaction—in which a user sends a request to a remote computer server, gets back a response, and the connection is broken—leaves the computer server with no history of the user's prior requests. NLM's solution for the "stateless" nature of normal Web interactions was to develop an Expert State Engine at the heart of the Internet Grateful Med gateway. This program has two parts: a "listener" that talks to a user's computer and an "expert state maintainer" that remembers what users have done and has rules for mapping terms and creating and refining searches.

For more information on this latest member of the Grateful Med family of programs or other aspects of accessing online databases via the Web, contact NLM's Internet Grateful Med development team (e-mail: access@nlm.nih.gov). ■

The Numbers Say It All

More than 125,000 individuals and institutions currently have accounts for searching the National Library of Medicine's 40 online databases. These users made more than 7.5 million searches in 1995. Users of the original Grateful Med do 90% of their seraching in Medline, which contains more than 8 million citations. More than 30,000 new citations are being added each month.

Metathesaurus Muscle

The National Library of Medicine's Unified Medical Language System (UMLS) Metathesaurus is an electronic Rosetta stone containing 589,000 names for 253,000 concepts in 30 biomedical vocabularies, thesauri, or classifications. Users of Internet Grateful Med draw upon this deep and carefully organized reservoir of medical terms when they employ the "find related" function, which compares the user's search terms with all terms in the Metathesaurus to produce a ranked concept "hit list," concept definitions, and Medical Subject Headling (MeSH) notes. Many of the concepts will be underlined, indicating that they are hyper links that the user can click on to ask Internet Grateful Med to create a graphic tree display of related MeSH terms. In another striking feature made possible by the Metathesaurus, Internet Grateful Med offers direct access to millions of pairs of co-terms, which are concepts that appear as "major topic" index terms for the same citation in Medline. With the single click of a mouse, users can include a concept and co-term—or even a triad of a concept, qualifier, and co-term—to improve their searches. ■

Leader of the Pack: Dideoxy Fingerprinting For Finding Unknown Mutations in Genes

Explosive progress in understanding the genetic basis of human disease and drug resistance in microorganisms has left researchers urgently in need of a method for routinely screening specific genes for mutations. A wide variety of different types of mutations may underlie the pathogenicity caused by changes in any given human or microbial gene so the exact mutations in the causative gene cannot be predicted ahead of time in most cases. Even investigators who are attempting to link a newly identified gene with a specific hereditary syndrome would be well served by having a simple way to check for the existence of any mutations before they begin the laborious process of sequencing the entire gene. Consequently, whether the complete gene is known or not, investigators would like to have a foolproof "mutation test." The new dideoxy fingerprinting (ddF) technique reviewed in this Hot Methods Clinic appears to have the sensitivity and ease of use sufficient to put it ahead of a small pack of other techniques in fulfilling this important research need.

Direct genomic sequencing, the gold standard for mutation analysis, is labor-intensive, expensive, and time-consuming. Several shortcut mutation-screening methods have therefore been developed. These include heteroduplex analysis, chemical mismatch cleavage, and denaturing gradient gel electrophoresis (1). Although faster and cheaper than direct genomic sequencing, these methods are technically difficult, labor-intensive, prone to false negatives or false positives, insensitive, and may require relatively large amounts of input template DNA.

The most widely used system for detecting point mutations has been single-strand conformation polymorphism analysis (SSCP) (2). SSCP starts with PCR amplification of target DNA. The amplified and partially denatured strands are then separated on a non-denaturing polyacrylamide gel. A mutation in the DNA strand generally causes a change in the three dimensional conformation, and the altered DNA migrates to a different point on the gel compared with the wild-type control. SSCP is simple to perform; unfortunately, however, it can miss many mutations (4), such as single-base substitutions of cytosine (C) to thymidine (T), that do not alter the 3-D shape enough to significantly change the migrational properties of the strand. In addition, SSCP will provide no information about the approximate location of the mutation within the screened segment.

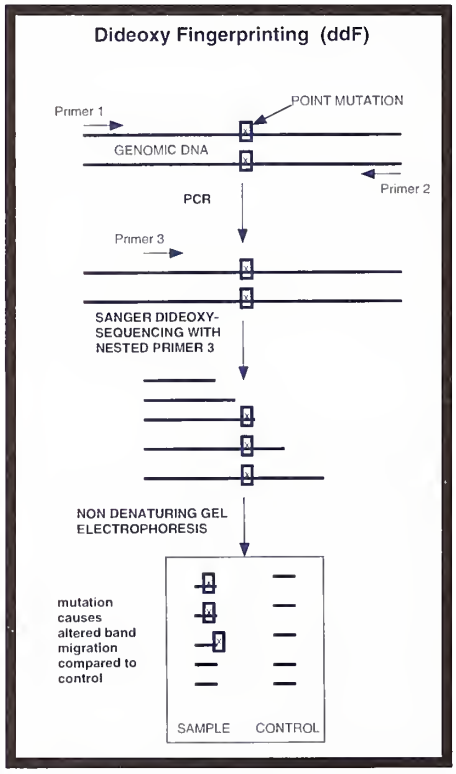


Figure 1. Schematic diagram of ddF.

The Method and How it Works

To overcome the drawbacks of SSCP, Sarkar et al. proposed ddF (3), which is a hybrid between direct genomic dideoxy-sequencing and SSCP analysis. The concept is simple: a standard Sanger sequencing reaction using only one dideoxy terminator is electrophoresed through a nondenaturing gel. In addition to detecting mobility shifts in the fragments containing the mutation, this method also picks up mutations that lead to a gain or loss of dideoxy termination bands (see Fig. 1). Thus, ddF is less influenced than SSCP by the nature of the mutation. ddF can detect mutations with close to 100% sensitivity up to 250 base pairs (bp) from the 5^1 end of the nested primer, and bi-directional ddF can screen a 500- to 550-bp region of DNA. By comparison, SSCP allows detection of between 70% and 95% of mutations for small PCR segments of 200 bp or less, and its sensitivity decreases rapidly with increasing size of the PCR product (3).

The superior performance of ddF was demonstrated by Teresa Felmlee and associates at the Mayo Clinic in Rochester, Minn., who compared it with SSCP on blinded samples of drug-resistant *Mycobacterium tuberculosis*. They found that prolonged electrophoresis time was required for discrimination of SSCP differences in strand migration compared with ddF. They also noted that some C-to-T transition mutations that were correctly identified by ddF could not be picked up by SSCP. Other researchers report that ddF detected 100% of p53 mutations in breast cancer (6) and all 84 different mutations, including all 12 possible types of base substitutions, in the human blood-clotting factor IX gene (7).

As we cross into the new millennium and the Human Genome Project approaches the identification of all human genes, the medical diagnostic lab will be transformed (8). While we wait for genetic testing to be miniaturized on a chip (8), methods such as ddF, or its modification, bi-directional ddF (7), will likely play a key role in finding mutations in hereditary-syndrome genes, in detecting drug resistant microorganisms, and in determining risk for acquired diseases. As a research tool, ddF will undoubtedly serve as a prominent mutation screening test that will aid in the linkage of newly identified genes to specific diseases.

Protocol

Mention of a specific commercial product or company does not constitute an endorsement.

1. To prepare the mutation-detection-enhancement (MDE) gel, swirl the

by Zsolt Orban, M.D., NIDDK;
A. Lee Burns, Ph.D., NIDDK;
Michael Emmert-Buck, M.D., Ph.D., NCI,
and Lance A. Liotta, M.D., Ph.D., NCI

following in an empty plastic gel-bottle: 35 mL of 2x MDE stock (manufactured by FMC Bioproducts); 4.2 mL of 10x Tris-boric acid-EDTA buffer; and sufficient distilled water to bring the total up to 70 mL. Mix in 280 µL of 10% ammonium persulfate and 28 µL of tetramethyl-ethylene diamine (TEMED). Pour a 0.4-mm-thick sequencing-type gel, pressing a sawtooth comb into the gel to create the wells. This comb gives results superior to those obtained with a shark-tooth comb. Store gel in a cold room (8 °C) and allow to set there for a minimum of 2 to 3 h. before removing the comb.

2. Prepare a stop solution with final concentrations of the following: 7 mol urea/L, 50% formamide, 3 mmol EDTA/L, and 0.5% bromphenol-blue-xylene cyanol.

3. Generate the amplicon to be analyzed by standard polymerase-chain reaction (PCR) using a proof-reading DNA polymerase. Store at -20 °C until used for fingerprinting.

4. The next step is to perform dideoxy fingerprinting on the amplicon with a nested, end-labeled primer, using your preferred T4 kinase labeling method. Design this primer so that its melting temperature is somewhat higher than that of the two primers used in the first PCR reaction. For a typical dideoxy fingerprinting reaction, mix the following: 6.0 µL distilled water, 2 µL of 5x Taq buffer; 0.1 µL of 2.5 mmol NTP/L; 0.2 µL of 10 mmol ddGTP/L; 2 pmol (1µL) of end-labeled primer; one unit of Taq polymerase; and 0.5 µL of the template DNA from the PCR reaction in step 3. Perform a typical cycle-sequencing reaction (30 to 40 cycles).

5. Add 50 µL of the stop solution, prepared in step 2 above, to the completed 10 µL of ddF reaction product. Incubate the samples at 90 °C for 5 min. and quick-chill on ice. Load a 3 µL aliquot onto the gel. Run gel at 20 Watts constant power until the xylene cyanol migrates two-thirds of the way down the gel. Subject the gel to autoradiography.

Trouble-Shooting Tips

These tips are adapted from Q. Liu, J. Feng, and S.S. Sommer (7).

1. Accurate pipeting is critical.

2. Use sawtooth combs yielding 32 or 64 wells for each gel.

3. Quick-chill the samples after boiling by immersing in ice water. This will reduce fuzzy bands.

4. Cool the gel or run at low temperature (8 °C in cold room).

5. For the fingerprinting, choose a dideoxy nucleotide that has a uniform spacing of termination segments, especially near the top of the gel.

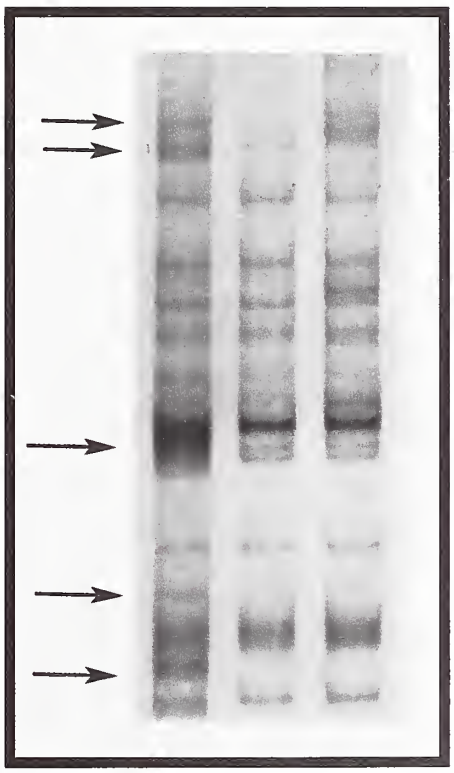


Figure 2. Example of a positive screen. *Arrows denote alterations in the band migration pattern. Lane 1: Mutation present. Lane 2: Control. Lane 3: Mutation present (different than in sample 1). Mutants were provided as a courtesy of Marcy Grace, NCHGR.*

6. Control experimental conditions closely. The fingerprint obtained is highly reproducible if run under identical conditions (e.g., temperature, wattage, and gel-preparation). Different running conditions, such as different wattage, can lead to altered sensitivity in picking up mutations and can produce different band-migration patterns.

7. Never score a sample as negative unless it is directly next to a control sample. To avoid false negatives, make sure the entire region of interest is represented in the gel.

8. Do not score a sample as positive if the intensity of the signal fades out as the segment gets larger. This pattern could be due to a poor termination reaction.

9. Assume that any segment of the SSCP migration that is clearly different from a normal segment in the flanking lane in the gel contains a mutation.

Contacts

Zsolt Orban, NIDDK
Phone: 402-7834
Michael Emmert-Buck
and Zhengping Zhaung, NCI
Phone: 496-2912
A. Lee Burns, NIDDK
Phone: 496-4616
Steve Sommer, Mayo Clinic
Sommer offers a kit of protocols
and reprints relating to ddF.
Phone: 507 284-4597, Fax: 507 284-3383

References

1. M. Grompe. "The rapid detection of unknown mutations in nucleic acids." *Nature Genet.* **5**,111–17 (1993).

2. M. Orita, H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekiya. "Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms." *Proc. Natl. Acad. Sci. USA* **86**, 2766–70 (1994).

3. G. Sarkar, H.S. Yoon, and S.S. Sommer. "Dideoxyfingerprinting (ddF): a rapid and efficient screen for the presence of mutations." *Genomics* **13**, 441–43 (1992).

4. Q. Liu and S.S. Sommer. "Parameters affecting the sensitivities of dideoxy fingerprinting and SSCP." In: *PCR Methods and Applications, Vol. 4.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 97–108 (1994).

5. T. Felmlee, Q. Liu, C. Whelen, D. Williams, S. Sommer, and D. Persing. "Genotypic detection of *Mycobacterium tuberculosis* rifampin resistance: comparison of single-stranded conformation polymorphism and dideoxy fingerprinting." *Jour. Clin. Microbiol.* **33**, 1617–23 (1995).

6. H. Blaszyk, A. Hartmann, J.J. Schroeder, R.M. McGovern, S.S. Sommer, and J.S. Kovach. "Rapid and efficient screening for p53 gene mutations by dideoxyfingerprinting." *Biotechniques* **18**, 256–60 (1995).

7. Q. Liu, J. Feng, and S.S. Sommer. "Bi-directional dideoxy fingerprinting (Bi-ddF): a rapid method for quantitative detection of mutations in genomic regions of 300-600 bp." *Hum. Mol. Genet.* (in press).

8. R. Nowak. "Entering the postgenome era." *Science* **270**, 368–71 (1995). ■

RECENTLY TENURED

Edward Berger *joined NIAID's Laboratory of Viral Diseases as a visiting scientist in 1987 and was recently appointed chief of the Molecular Structure Section. He received his Ph.D. in biochemistry from Cornell University in Ithaca, N.Y., in 1973 and served on the faculty at the Worcester Foundation for Experimental Biology in Massachusetts from 1977 to 1987.*

My laboratory focuses on the interactions between enveloped viruses and their cellular receptors, with particular emphasis on the human immunodeficiency virus (HIV). Our overall goals are to elucidate mechanisms by which virus-receptor interactions lead to fusion and entry, to understand how such interactions contribute to viral pathogenesis, and to use this knowledge to develop novel therapeutic strategies to treat viral infection. My initial studies concerned structural analysis of CD4, the primary HIV receptor. In collaboration with other lab members, I localized the region of CD4 involved in binding to gp120, the external subunit of the HIV envelope glycoprotein (env). My group then went on to characterize CD4-induced changes in the structure of env and the possible roles of discrete regions of CD4 in the fusion process.

On the basis of our structure-function analyses of CD4, we devised a novel therapeutic strategy for the targeted killing of HIV-infected cells. With NCI collaborators, we genetically engineered a chimeric toxin so that it contains a portion of CD4 linked to the active regions of *Pseudomonas* exotoxin A. This drug, CD4-PE40, kills HIV-infected cells with extremely high selectivity and potency in vitro. Unfortunately, clinical trials at NIH and several other U.S. centers revealed unexpectedly high liver toxicity in HIV-infected patients, and no benefit occurred at tolerated doses. However, other applications are very promising. For example, several investigators who are developing gene-therapy protocols are using CD4-PE40 ex vivo to eliminate HIV-infected cells before genetically altered cells are reintroduced to the patient.

Using recombinant vaccinia virus technology, my lab developed a highly versatile reporter-gene assay system for quantitation of fusion between env-expressing and CD4-expressing cells. We are using this assay to probe mechanistic features of fusion mediated by the HIV env and have extended our studies to paramyxoviruses such as measles virus and respiratory syncytial virus. The assay is also a valuable tool for the rapid, quantitative screening of antiviral drugs and antibodies that block the fusion step of virus infection.

A major focus of our mechanistic work concerns the specificity of the fusion processes mediated by the interaction between HIV env and CD4. Studies from several groups, including mine, have indicated that CD4 must be expressed on a human cell in order to support fusion by the HIV-1 env. Working with NCI investigators, we demonstrated that this restriction is due to the requirement for an unidentified human-specific fusion accessory component of the CD4-expressing cells. A related problem concerns the marked tropism of different HIV-1 isolates for infection of human T-cell lines vs. primary macrophages. My group recently showed that this cytotropism is due primarily to the fusion specificities of the corresponding envs. Our subsequent results suggest that T-cell-line vs. primary macrophage tropism is due to the requirement of the corresponding envs for distinct fusion-accessory factors differentially expressed in various CD4-positive target-cell types.

A major goal of our work is to identify these accessory-fusion factors. To this end, we used the vaccinia-based reporter-gene assay system in functional screening of cDNA libraries. We isolated a cDNA encoding a seven-transmembrane segment G protein-coupled receptor, which has the properties expected for a fusion accessory-factor for T-cell-line-tropic HIV-1 isolates. The identification of a new molecular player in the fusion process opens major new directions for our mechanistic structure-function studies.

Tom Schwan *received his Ph.D. in 1983 from the University of California at Berkeley, where he studied parasitology and medical entomology. In 1986, he joined NIAID's Rocky Mountain Laboratories in Hamilton, Mont., where he is in the Laboratory of Microbial Structure and Function.*

My laboratory investigates bacterial pathogens transmitted by ticks and fleas. We focus on the Lyme disease spirochete, *Borrelia burgdorferi;* a relapsing fever spirochete, *Borrelia hermsii;* and the plague bacillus, *Yersinia pestis*. My early work focused on developing rapid diagnostic techniques to detect these agents in their respective tick and flea vectors. We also applied recombinant techniques to clone and express genes of *B. burgdorferi* that would be useful in the serodiagnosis of Lyme disease. One recombinant antigen, P39, has been patented and developed commercially into several diagnostic test kits for testing human serum samples for specific antibodies associated with Lyme disease. We also produced the first recombinant-based vaccine for plague.

More recently, we have begun to examine how these bacteria adapt and change during their infections in their arthropod vectors. For this, we rear and maintain live colonies of several species of ticks, including *Ixodes scapularis* and *Ornithodoros hermsi,* the respective tick vectors of Lyme disease and relapsing fever spirochetes. We also maintain a colony of the Oriental rat flea, *Xenopsylla cheopis,* for our work on plague.

The relapsing fever spirochete, *B. hermsii,* contains at least 40 genes that encode variable major proteins (Vmps). At any given time, only one gene is expressed by the spirochete and each Vmp confers serotype specificity on the bacterium's surface. In humans and other animals, these genes form the basis of the spirochete's antigenic variation, allowing the organism to temporarily evade the mammalian host's humoral immune response to infection.

We are investigating the antigenic behavior of relapsing fever spirochetes during their infection in the tick vector and how the different serotypes affect transmission by ticks. By infecting different cohorts of ticks with different serotypes of *B. hermsii,* we have found that the spirochetes do not change antigenically while they are in ticks. Hence, ticks transmit the same serotype that was ingested during a previous blood meal. However, the serotype has a striking influence on the frequency at which spirochetes are transmitted when the infected ticks feed again. This means that one serotype is rarely transmitted, whereas another is transmitted during one out of every three tick feedings. We are now trying to understand the basis for such differences.

The closely related Lyme disease spirochete causes more human infections in the United States than all other vector-borne illnesses combined. Recently, we demonstrated that during the spirochete's residency in the tick midgut, its outer surface changes as the tick attaches to a mammalian host and ingests blood. An increase in temperature is also involved in the spirochete's synthesis of new proteins during the feeding, and it corresponds to the time at which these bacteria escape the tick's midgut, infect salivary glands, and are transmitted via the saliva. Such changes have important implications for both diagnostics and vaccine development. We hope that our studies with both species of spirochetes and their respective tick vectors will allow us to identify factors responsible for tick-spirochete specificity and critical events in the transmission of spirochetes to humans. ■

SPEAKING AND WRITING ABOUT SCIENCE

by Rebecca Kolberg

Protein purification doesn't faze you. You don't blink at running an in vitro transcription reaction. But when it comes time to present your results in a paper or a talk, you break out in a cold sweat. Yes, you are among the legions of researchers who could use some help in learning how to write and speak about your science in the most effective way.

"Most scientists receive little or no formal training in writing and consider writing the last—and most odious—part of their work, rather than an integral part of the research process," says Ruth Guyer, the Ph.D. immunologist and veteran science writer who taught NIH's initial offering of the "Writing About Science" course from Jan. 24 through Feb. 14.

Even though the four-week course, sponsored by the Office of Science Education and the Office of Research on Women's Health, required 12 hours of class time plus a tough load of writing and reading assignments, more than 160 NIH researchers signed up for the 16 slots. The writing course is scheduled to be held again in May and September. A five-week companion course, "Talking About Science," taught by actor and speech coach Scott Morgan, was getting under way as *The NIH Catalyst* went to press. The speaking course, which will be held again in June and October, covers a range of public-speaking situations encountered by scientists: introducing host speakers, fielding questions, talking at a poster session, and delivering a 10-minute scientific presentation.

Gloria Seelman

Course participants Stefano Bertuzzi, left, Patricia Cortazar, Peter Balint-Kurti, and Yvette Miller

Unlike some general science-writing courses offered at universities and the Foundation for Advanced Education in the Sciences, the new course specifically seeks to hone biomedical researchers' skills in writing articles suitable for publication in peer-reviewed scientific journals. Most assignments are aimed at writing papers based on participants' current research—from choosing a target journal to putting the finishing touches on the crucial abstract. Researchers are also encouraged to place themselves in the shoes of journal editors and peer reviewers through exercises such as critiquing their classmates' papers.

Yvette Miller, a senior staff fellow in the Clinical Center's Department of Transfusion Medicine, says the course helped steer her in the right direction for her first major scientific paper, which features results of her work on transplants of stem cells from umbilical cords. "It definitely makes the writing process less traumatic for you and your mentor," says Miller, who also plans to follow Guyer's advice that researchers write up their results as they go along, rather than waiting until all experiments are done to begin working on a paper.

"Writing helps you make sense of what you're doing and build a better framework in which to place your experiments," says Guyer, who in her days at the bench at the University of California at Berkeley discovered that when she conducted writing and research in tandem, she could detect—and repair—holes in her experimental designs *before* her data were subjected to the scrutiny of peer review.

Although every scientist has strengths and weaknesses when it comes to writing, Guyer says the most common problem is overuse of jargon. "You don't want to make the reader struggle to navigate through your writing," she says. Many scientists also weaken the impact of their message by using words or phrases that are unnecessarily long. For example, Guyer says replacing the three-syllable word "utilize" with the one-syllable word "use" makes for a more direct—and more powerful—sentence.

In their course evaluations, the students, about half of whom were M.D.s and half Ph.D.s, solidly supported continuing the course. One even suggested that NIH establish a writing clinic where researchers could go for help as they write papers. Most also said they would recommend the course to other researchers. "Go for it!" one participant wrote, adding, "Have a mini-paper topic ready to write up. Be prepared to work hard and to learn to accept useful criticism."

For more information on the writing and speaking courses, contact Gloria Seelman at the Office of Science Education (phone: 496-0608; fax: 402-3034; e-mail: gq5@cu.nih.gov). ■

Trimming Down The Bulk Mail

We all know what it's like to find our mailbox stuffed with ad after ad for products we don't use, and those of us who work in offices have the additional annoyance of getting multiple fliers and catalogs addressed to staff who left NIH long ago. Unwanted bulk mail also constitutes a major burden and expense for the NIH Mail Service, and throwing it out is environmentally unfriendly and contributes to waste-disposal costs. To make it as easy as possible to stop unwanted bulk mail at its source, NIH has developed a new postcard that staffers can use to request cancellation of undesired mailings. All you need to do is tape or paste the address label from unwanted mail on the card, address the card to the sender, and drop it in the mailbox. Vendors will probably be glad to save some money (and do the environment a favor) by canceling mailings of unproductive sales literature. Result: less "junk" mail to clutter scientists' lives and wastebaskets. To get a packet of postcards, ask your administrative officer for NIH Form 2759, "Request for Deletion from Mailing List," or call customer service at the NIH Mail Services Branch (496-3586). For an introductory period, the cards—but not the postage—will be provided free of charge. Get 'em while they last! ■

—Jane Sayer, NIDDK

NIH's ARK
continued from page 1.

In addition to more efficient use of space and staff, other cost-cutting measures implemented by Lang include coordinating the purchase of commonly used supplies. Previously, each VRP building ordered its own supplies. Lang is also taking a hard look at the environments in which the animals are kept to see whether some relatively expensive "containment" housing, which provides each animal cage with its own filtered air supply, can be replaced with less costly conventional housing that has a common air supply for a whole room of cages.

But housing is far from the only service that VRP can provide. VRP manages a repository of more than 350 strains of genetically defined rodents and rabbits for distribution to researchers and also assists intramural researchers in selecting appropriate models and in characterizing strains. It can also help researchers procure appropriate animal models from commercial breeders. In addition to monitoring animal colonies for infectious disease and pathogenic entities, VRP's veterinary staff can perform or assist NIH researchers with radiographic procedures and experimental surgery at the program's centralized facilities.

NHLBI's Daisy Lazarous, who, as part of Ellis Unger's team, has interacted with VRP on preclinical studies of angiogenesis for ischemic heart disease, says the VRP staff in general, and veterinarians John Bacher and Victoria Hampshire in particular, "have been extremely crucial in our ability to carry on our animal studies and collect meaningful data."

"There never has been a problem in scheduling surgery using Dr. Bacher's facilities—however intense the pace, in using radiology facilities, or in postoperative care by Dr. Hampshire's staff," says Lazarous, who, in contrast, has often encountered problems scheduling the use of radiology and surgery facilities through other animal services. Lazarous credits the free exchange of ideas for the good working relationship that her group has developed with VRP staff. "They give us adequate feedback regarding our protocols and problems and, in turn, are very amenable to suggestions from us. "

Still, as Lang notes, many intramural researchers remain unaware of the wide array of expertise and technology available at VRP. He cites the example of an intramural scientist who originally planned to perform thymectomies on monkeys. After talking with VRP veterinarians and reviewing their facilities, the researcher revised his experimental design and opted for a nuclear-medicine scan and surgical biopsy—a sophisticated approach that yielded more informative data.

Cryopreservation and construction of transgenics are other areas where VRP is helping to keep NIH on the cutting edge of biomedical science. VRP recently expanded the availiability of its animal-embryo cyropreservation facilities (see box, page 13), and the program's staff is also currently

Thoughts of a Veteran Vet


Lorna Heartley

Victoria Hampshire

To a researcher in the throes of a crucial animal study, veterinarians and the animal-care rules they enforce may seem like roadblocks to scientific discovery. But that doesn't have to be the case. Scientists who have taken a little time to understand the veterinary profession often count vets among their most valuable allies in their quest to collect solid data.

To provide NIH investigators with a vet's perspective on research, *The NIH Catalyst* talked with Victoria Hampshire, D.V.M., who has been a vet with NIH's Veterinary Resources Program since 1989 and is currently head of its carnivore/ungulate unit and conventional rodent/rabbit unit. Hampshire offered scientists this advice on getting the most out of their relationships with animal-care vets.

- Be aware that in the United States, vets are highly trained professionals, with four years of sophisticated graduate training and often a residency in a specialty area.
- Keep in mind that vets generally have a better understanding of animal anatomy and physiology, particularly of non-rodent species, than do M.D.s or Ph.D.s.—an understanding that may prove helpful in designing experiments or performing surgery.
- Remember that animal-care vets are required by law to uphold the Animal Welfare Act, which are rules passed by Congress and which cannot be changed by individual vets.
- Contact vets *early* in the protocol-development process. Their input on types of animals, procedures, drugs, and staff to use can save time and money further on down the road. Vets should also be able to furnish you with a realistic estimate of what the study should cost.
- Recognize the ways vets and their strategies can improve your data collection. For example, providing better pain medication, monitoring devices, and even animal companionship can substantially improve postoperative survival, thereby providing scientists with more—and more uniform—data.
- Give credit where credit is due. Make vets co-authors of papers if they make substantive original contributions, or mention their contributions in the acknowledgments section. ■

—R.K.

experimenting with the technology needed to make transgenic pigs.

However, not all the changes on Lang's agenda are high-tech. "We need to enhance communication. Everybody here is busy, so we tend to focus on our own little world. VRP staff needs to be proactive and reach out to investigators," says Lang. Toward that end, the VRP director has asked his staff to go to investigators' labs and meet with them face-to-face whenever possible, rather than just reaching for the phone or firing off an e-mail message. Veterinarians are also being encouraged to attend lab meetings and seminars on topics of projects in which they are involved. "The more we learn about investigators' projects and research areas, the better service we can provide," Lang says.


Lorna Hearley

C. Max Lang

Comments and suggestions from researchers are another form of communication that Lang wants to encourage. "If they [researchers] have questions or if they have a problem, they should let us know. Sharing information is a crucial part of research," he says. "'If we don't know their concerns or problems, there's nothing we can do about it."

For more information on VRP's services and resources, contact Lang at 496-2527. ■

Mice on Ice

After years of having to turn away NIH researchers who were seeking help with creating and storing of frozen animal embryos, the Veterinary Resource Program's cryopreservation facility is opening its freezer doors to the intramural research community.

William Rall, a Ph.D. physiologist who took over the leadership of the Embryo Cyropreservation Program in January, says his facility now has sufficient staff and resources to move beyond the important task of preserving mouse and rat embryos for VRP's National Genetic Resource and start providing similar services to intramural investigators.

"We are now able to assist NIH scientists with all aspects of embryo-related services," says Rall. "We are prepared to do whatever is needed to help them, including going to their labs to collect and cryopreserve embryos for storage at VRP or obtaining and breeding the genotype in our animal facilities to produce the embryo-donor females."


Bill Branson

Embryo cryopreservation, which was first performed in 1973 and which VRP began in the early 1980s, is used for two main purposes: to store infrequently used animal models and to provide insurance against the loss of valuable models that are in constant use. Maintaining models as frozen embryos is a cost-effective way to manage animal models regardless of whether they were produced by conventional breeding or by transgenic or knockout procedures. According to Rall, the cost of cryopreserving a mouse embryo for decades or more has been estimated to be equal to the cost of maintaining the live animal for just one year. Even if a researcher chooses to maintain a small breeding colony of a special animal model, a bank of frozen embryos provides a safety net if the model becomes compromised by genetic changes, disease, or breeding failure.

Embryo cryopreservation can also be used along with embryo transfer to eliminate disease that may undermine research projects. Embryos can be collected, washed free of contaminating viruses or microorganisms, and transferred aseptically into a disease-free surrogate mother. Rall notes that the logistical complications that arise from collecting and transferring embryos on the same day can be avoided by cryopreserving the embryos after the washing step and transferring them at a later date.

Although a lab might opt to purchase its own liquid nitrogen tanks and other equipment needed for cryopreservation at a cost of about $15,000, Rall warns that the biggest problem is training technicians. "What happens is the lab tech becomes proficient after several months of experience and then leaves," he says. "However, [if] the new tech doesn't know that the liquid nitrogen containers need to be filled, then everything thaws and all is lost."

In addition to routine embryo collection, cryopreservation, and storage, the cryopreservation facility staff is available to help NIH researchers with more complex problems. For example, Rall says his group is currently assisting a senior NCI investigator who wants to import a transgenic mouse model from Japan as frozen embryos. Upon arrival at NIH, the embryos will be thawed and transferred into a disease-free surrogate mother. Work is also beginning on the cryopreservation of embryos from guinea pigs and hamsters. ■

—R.K.

Stored Tissue
continued from page 1.

relatives through loss of health insurance, compromised employability, or psychosocial trauma. Therefore, health-care providers and researchers alike support safeguarding the confidentiality of test results. Conflict arises over how to protect the individual without compromising biomedical research that may benefit society as a whole.

Currently, human subjects are protected from invasion of privacy and other potential hazards of research by federal regulations put into place in 1981 by the Department of Health and Human Services' Office for Protection from Research Risks (OPRR). Under OPRR rules, all institutions that receive federal funds must establish Institutional Review Boards (IRBs) to review research involving humans and to stress the obligation to obtain informed consent.

Importantly, OPRR guidelines draw distinctions among *anonymous* samples, which are never labeled with identification that could link the specimen to a person; *anonymized* samples, which are rendered anonymous by irreversibly removing identifiers; *identifiable* samples, also referred to as linked, linkable, or coded samples, which are unidentified for research purposes, but can potentially be linked to the source through use of a code; and *identified* samples, which are labeled with a specific patient identifier such as a medical record number. IRBs are asked to consider a research proposal's potential risk to human subjects and whether it involves retrospective or prospective samples, and then, based on that information, to stipulate the type of informed consent to be used. Currently, research on retrospective, anonymous samples usually does not require IRB review because it is thought to pose little risk to human subjects. However, prospective research on identified samples must undergo IRB review and requires specific informed consent due to the risk it poses to a subject's privacy. Between these extremes are gray areas—and it is these gray areas that the recent recommendations address.

New Twists On Old Questions

by Leslie Biesecker, M.D., NCHGR

The current controversy about genetic research on stored specimens may be partly attributable to a misunderstanding that higher standards are being proposed for genetics research. In fact, what is being suggested is that current practice needs to be brought into line with existing regulations.


Lorna Heartley

Leslie Biesecker

The informed-consent issues for stored samples are not unique or novel but they do represent new twists on old questions. In addition, the history of research on stored specimens is one of a gradual evolution from clinical care and diagnosis toward exploratory research. This evolution was not always accompanied by a corresponding evolution in protections for human subjects.

My reading of the NCHGR-CDC workshop document is that it applies existing standards to activities that are currently being performed with inadequate or no informed consent. In a study published in *IRB: A Review of Human Subjects Research*, Robert Weir and Jay Horton of the University of Iowa found that only 23% of a nonrandomly selected group of 103 consent forms explicitly requested permission to bank specimens when such activity is taking place. Although these data do not directly address the use of previously collected specimens for research, they suggest that current practice is not uniform when it comes to consent for molecular genetics research.

It is fundamental to the ethical conduct of research that people be respected as autonomous agents. It is unacceptable by any reasonable standard to involve people in research without their consent (or the consent of their appropriately designated surrogate) when that research can have adverse effects on them. That this requirement poses formidable challenges for stored specimens is certain. What is also certain is that there are creative mechanisms for acquiring specimens and upholding autonomy. Many studies on allele prevalence can be conducted by stripping samples of identifiers. This stripping separates the sample from its source and insulates the source from adverse events. Other studies that require correlation with clinical characteristics can be performed by linking a small amount of clinical data (insufficient to identify the sample) and then removing the identifiers. This approach requires careful hypothesis generation and statistical analysis to ensure adequate power to address the hypothesis and inadequate power to deduce identities from the clinical data. Granted, this approach is not amenable to small-scale "fishing expeditions" that occasionally identify important scientific avenues but such expeditions, in my opinion, are more often fruitless and wasteful.

Another solution is to develop banks of tissue for the express purpose of research. These banks could be prospectively collected with full informed consent for research into a particular area (e.g., breast cancer pathogenesis) and associated with a broad array of clinical data that would be useful for many future studies. The consent process can be streamlined by designing the bank to be used without the return of individual results to the subjects. Establishing research banks would initially be expensive, but this investment would be amortized by repeated use of the specimens and would obviate redundant clinical ascertainment. In addition, it is likely that a highly organized and prospective bank would contain better quality data than would less organized specimen acquisition by individual researchers.

Patients should have ongoing reassurance that all activities surrounding their clinical care are solely directed toward individual benefit. Patients must be informed and given a choice about whether to become research subjects. Just as importantly, research into the molecular etiology of disease must continue because of the enormous benefits to the public good. There is a clear difference between research and patient care despite arguments that blur the issues. Informed consent is crucial for maintaining a wall that separates clinical activities from research activities in order to avoid loss of confidence in clinical providers and to maintain the stature of the research enterprise. Erosion of that separation may lead to short-term gains for some research projects, but it will result in loss of trust in both researchers and clinicians and put our social consensus for biomedical research at risk.

Use vs. Misuse

by Mark E. Sobel, M.D., Ph.D., NCI

Although the following views are my own, they have been greatly influenced by my participation in an ad hoc committee of pathologists that was formed to respond to the "Genetic Privacy Act." Our goal is to seek a consensus that, while being respectful of informed consent, would not compromise patient care or unduly encumber research that is relevant to human disease.

Recent proposals to enforce confidentiality policies and to restrict genetic testing and research unless the subject gives specific informed consent for each test have a laudable goal: protecting the privacy and autonomy of human subjects. However, if not worded carefully, such policies


Lorna Heartley

Mark E. Sobel

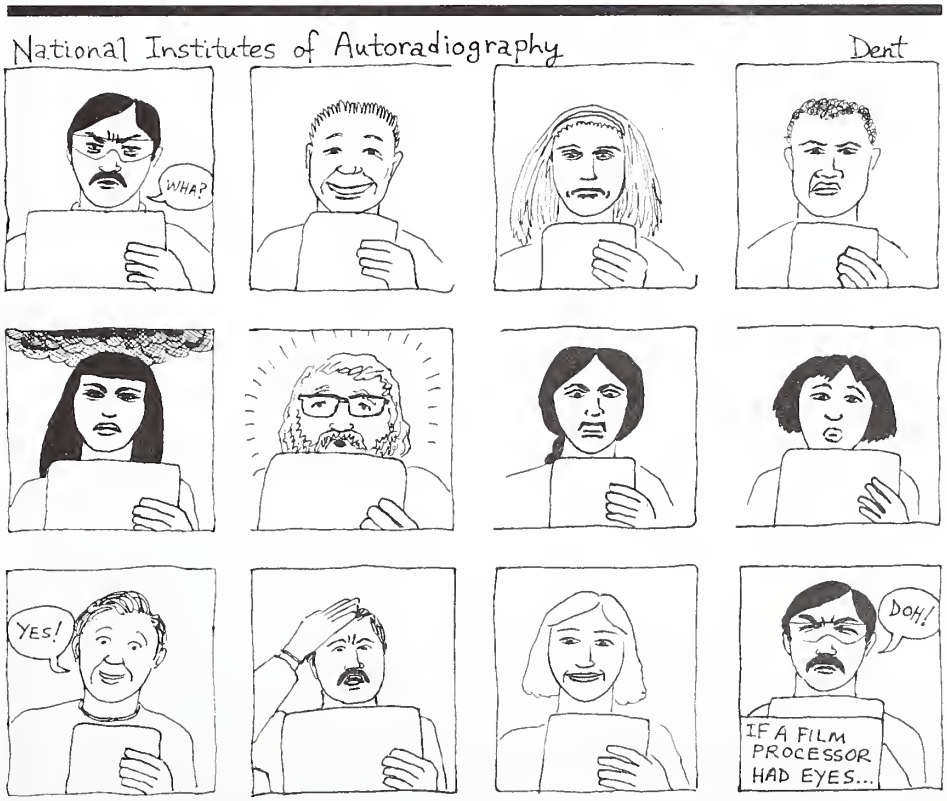
might unintentionally conflict with pathologists' provision of diagnostic services, thus impairing patient care. Some proposals define a genetic sample as any tissue or bodily fluid from which DNA can be extracted, including urine and sputum. In addition, a patient's genetic status can be detected by tests that do not directly assess nucleic acid structure, such as protein, immunologic, biochemical, and morphologic tests. Under the broadest interpretation of some proposals, any diagnostic test is potentially a genetic test—creating a liability nightmare for diagnosticians.

Efforts to strengthen informed-consent guidelines seem to have concentrated on prospective tissue banks in which people volunteer samples exclusively for research use. There is little disagreement that informed consent is necessary and relatively easy to obtain in such a setting. Volunteers can be offered an array of options, including designating use of their samples for specific research studies, keeping samples anonymous, and requesting test results or counseling. However, a large proportion of research is currently performed on residual tissues that were routinely collected with general consent during medical care. Access to these "leftover" tissues has been critical to the advancement of medical knowledge. Nonetheless, some proposed and recently enacted state legislation offers patients the option of having their tissue destroyed after appropriate tests are completed. Other proposals would apply the same informed-consent procedures developed for tissue banks to residual-tissue collection. These complicated procedures may intimidate patients who are awaiting surgery. Even under the best of circumstances, it is impossible to anticipate all future uses of residual tissue. In these cases, general consent should be sufficient with the proviso that all research results remain confidential.

Both the Genetic Privacy Act and the NCHGR-CDC workshop recommendations suggest that IRBs review research proposals before samples are anonymized and also determine whether subjects consented to the research at the time the samples were collected. If not, researchers might be required to recontact patients to get informed consent. Also, IRBs would be asked to determine whether researchers can collect their desired data by using a protocol that allows for informed consent. Currently, federal informed-consent regulations do not apply to tissue samples taken from individuals who have since died. However, the NCHGR-CDC workshop statement recommends that informed consent be obtained from the dead person's heirs and/or legal executors before new research is performed. These added requirements to recontact subjects or families would significantly increase the cost and time of performing research—a heavy blow in an era of shrinking research dollars.

A current advantage of using anonymous samples for genetic research is the exemption from obtaining informed consent. However, the inability to obtain correlative information from the medical record (such as patient outcome) might severely compromise utility of the data. Therefore, many research projects today use so-called identifiable, or linked, samples. Although such samples are not readily identifiable by the casual observer, a code exists that can enable researchers to link the sample to the donor. Under what circumstances might research on identifiable samples be eligible for the minimum informed-consent procedure? We cannot dismiss the possibility that an ethical dilemma can arise when a linkable sample is used in research with only minimal informed consent. For example, while examining tissue for a research study, a pathologist might find a previously undetected cancerous lesion. Isn't there an ethical obligation to contact the patient? One solution being considered by the pathology community would include the following provisions: the code that links the sample to the patient's identity would be physically separated from the research setting, researchers must demonstrate that they enforce confidentiality policies, researchers must provide written acknowledgment that no one involved in the research will attempt to gain access to the patient's identity or any other information in the medical record unless approved by the IRB, and the IRB will determine the best course of action should it agree that patient contact is necessary.

It seems to many pathologists that the fundamental issue concerning stored tissue samples is not the genetic information obtained from such samples, but its use or misuse. Although authors of the Genetic Privacy Act and the NCHGR-CDC workshop statement argue that the consequences of misuse of genetic information are worse than the misuse of other medical information, the operative word to me is "misuse." As long as confidentiality is strictly maintained, the need for specific informed consent for retrospective research on residual, archived tissue does not serve a legitimate purpose and could encumber scientific research. ■



CATALYTIC REACTIONS

In this issue, we are asking for your reactions in four areas: chemistry, Just Ask, Hot Methods Clinic, and scientists' retirement. **Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: catalyst@od1em1.od.nih.gov; fax: 402-4303; or mail: Building 1, Room 334.**

In Future Issues. . .

- Chemistry at NIH, A Dying Art?
- Alternative Medicine's Intramural Foray
- Hot Methods: DNA, All Strung Out
- Retirement, When Should Scientists Call It Quits?

1) We are working on an article about chemists at NIH. What role do you see for chemists in today's biomedical research environment? How do you think NIH in general has treated the chemistry profession?

2) In our new "Just Ask" column (see page 3), we are trying to find answers to scientists' questions concerning intramural research. What specific issues or problems would you like us to tackle?

3) What suggestions or comments do you have about the dideoxy fingerprinting technique featured in this issue's Hot Methods Clinic? What methods would you like to see covered in the future?

4) In a future issue, we plan to address the topic of scientists' retirement. When do you think scientists should retire? Do you think there should be a mandatory retirement age or a mandatory productivity level?

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 334, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; e-mail: catalyst@od1em1.od.nih.gov

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
Building 1, Room 334
Bethesda, Maryland 20892

FIRST-CLASS MAIL
POSTAGE & FEES PAID
DHHS/NIH
Permit No. G-763